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History of Avian Medicine in the United States. X. Control of Coccidiosis

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Fifty years ago, coccidiosis was probably the most feared disease of advanced poultry producers of that day. Because of the discovery and use of preventive chemotherapy, that fear is no longer all-consuming. However, improving methods for controlling these protozoan parasites remains high on priority lists requesting new research from the poultry industry. Severe mortality losses have become less frequent, but decreased production efficiency continues to produce morbidity losses due to coccidial infections. The industry has found that use of an anticoccidial drug is generally cost-effective as insurance against losses. However, the recurring expenditure for feed medication in large-scale broiler production reminds producers that control of this disease will be a continuing expense unless some cheaper method of control is discovered.

Clinical coccidiosis is now recognized as a problem associated with growing large numbers of birds in limited areas. Confinement permits the rapid accumulation of the large numbers of oocysts required to produce clinical coccidiosis. Harmless subclinical coccidiosis is more often the rule in small backyard or range-managed flocks. A few omnipresent oocysts may actually be beneficial in stimulating a gradual buildup of protective immunity. Broiler flock size in the 1930s was seldom greater than a few hundred birds in a single house. By the 1950s, flock size had increased to a few thousand. In the 1980s, many broiler houses had a capacity of 100,000 or more. The production economics of this expansion has benefited the consumer by decreasing the cost of poultry meat, making it more than competitive with the red-meat industry. Dramatic increases in flock size would not have been possible without the use of anticoccidial drugs.

Prevention and protection against coccidiosis through the use of medicated feed represent a unique approach to the control of a poultry disease. Because large quantities of drugs and advance planning are required, selection of a specific anticoccidial is now made in conjunction with feed-mill management.

This history addresses some of the developments in coccidiosis control that have permitted the poultry industry to expand and still live with this disease complex. Only a limited number of the hundreds of papers, reviews, or presentations made at numerous coccidiosis conferences can be cited in this history.

THE BIOLOGY OF THE COCCIDIA

The basic understanding of coccidiosis as a disease caused by several species of *Eimeria* protozoa came from publications of two poultry pathologists between 1910 and 1938. Although their laboratories were located across the continent from each other, the contributions of W. T. Johnson and E. E. Tyzzer as individuals or, in some cases, with cooperative publications laid the foundations for understanding coccidiosis as a disease complex involving several distinct species of coccidia. Tribute to their work was published by Lund (80) in number IV of this historical series. Study of their work is basic to understanding poultry coccidiosis. Recent research workers, thinking they may have made a significant new discovery, have often found in reviewing the literature that W. T. Johnson or Ernest Tyzzer had published similar findings many years ago.

Johnson, a professor of poultry pathology at Oregon State University, died in 1937 at the age of 45, at the height of his scientific career. Some of his basic discoveries on immunity to coccidiosis (62,63) were published posthumously by his wife, Mrs. W. T. Johnson, and William A. Schoenfeld, dean and director of the Oregon Experiment Station (64).

Tyzzer, a George Fabyan professor of comparative pathology at Harvard University, died

in 1965 at the age of 90. Besides his leadership in coccidiosis research, he is also recognized for his clearcut exposition on the life history of histomoniasis (blackhead disease) in turkeys and chickens. His series of drawings and descriptions of six species of coccidia are often copied in present-day textbooks (112,114). He shared research results with Johnson and generously gave him posthumous credit for description of two species of *Eimeria*. Their combined discoveries may be summarized in six basic principles that outline the distinctive characteristics of coccidiosis in chickens.

1. Pathogenicity and oocyst numbers. The degree of pathogenicity is directly affected by the number of oocysts ingested by the host at one time. Numbers in the thousands produce the characteristic signs of clinical coccidiosis with intestinal lesions, sloughing of the mucosa, and expulsion of mucous and/or blood, depending on the species involved. Less than 100 oocysts produce subclinical infection that can be detected only by indirect laboratory studies. As demonstrated by Johnson (61) and P. P. Levine (74), subclinical infections are so insidious and markedly different from the clinical disease that Norman Levine (71) has coined the term coccidiasis to contrast with clinical coccidiosis. Subclinical coccidiosis is common, yet it goes so unrecognized by many clinicians and poultrymen that they are often surprised to find that mild infections have occurred unnoticed in many flocks reared on range or in litter pens.

2. Species of coccidia. When Johnson and Tyzzer began their investigations, a parasite named *E. avium* was designated as the cause of several ill-defined intestinal diseases. The condition was sometimes attributed to coccidia, to another protozoan, *Histomonas* sp. (now known as the cause of blackhead), or to several poorly defined, bacterial-induced diseases. Together, these men established the concept that coccidiosis in chickens is caused by several distinct species of coccidia. Each species differs in the degree of pathogenicity, the number of oocysts required to produce tissue disruption, the host tissues that are parasitized, and in their life histories. Their findings required establishing and describing several distinct species.

Tyzzer discouraged a prevalent taxonomic practice of naming a new species of coccidia on the basis of an oocyst description alone (113). Valid species descriptions should include measurements, drawings or photographs and descriptions of all parasitic and free-living stages of the life cycle. Immunity induced in the host should indicate little, if any, cross-resistance with other described species derived by cloned cultures made from single oocyst isolations.

Classic descriptions and drawings were made of six species from the chicken (112,114). Absence of cross-infection was demonstrated among these species and others described from turkey, quail, and pheasant hosts. With chicken coccidia, Tyzzer accepted and extended the species description of *E. tenella* as described in 1891 by the French investigators Railliet and Lucet (94) as the cause of cecal coccidiosis. Tyzzer made the original descriptions of *E. acervulina, E. maxima,* and *E. mitis.* Tyzzer also accepted and added drawings to Johnson's oral description of *E. necatrix* and *E. praecox.* This publication provided assurance that Johnson's name would always appear as the original describer. *E. hagani* (1938) and *E. brunetti* (1942) were described by P. P. Levine (72,76) and *E. mivati* (1964) by Edgar and Seibold (32). The nine species from the chicken have often been recognized in the United States, and their presence has been verified in other parts of the world. The validity of *E. hagani* and *E. mivati* is currently being questioned by Shirley *et al.* (105). Unfortunately, current techniques of cryopreservation were unknown at the time these initial descriptions were made. No subcultures of the original isolates are now available for verification.

As many as seven species of *Eimeria* coccidia have been described from turkeys. Other avian hosts are parasitized with distinctive species belonging to the genera *Eimeria* and *Isospora.* Because each species of coccidia usually parasitizes only one host species, many early reports indicating cross-infections between different hosts are now considered erroneous. Another genus, *Cryptosporidium,* originally described by Tyzzer (111) from the mouse, contains species commonly parasitizing chickens, other birds, mammals, and humans. Because pathogenicity and species characteristics with its minute oocysts are currently under intense investigation, no further review of this group is included here.

3. Coccidiosis is a self-limiting infection. The term infection with bacteria and viruses usually implies unlimited multiplication after

the infective agent has gained entrance into the host. However, coccidial infections with *Eimeria* species usually stop reproducing if reinfection by a new crop of sporulated oocysts is prevented. After completion of the asexual cycles (usually two), multiplication and oocyst production cease. Reinfection occurs only after daughter oocysts have been released in the litter and have sporulated. Clinical coccidiosis occurs only after oocyst numbers have been increased to provide a heavy dosage, which usually requires completion of two or more life cycles.

4. Development of immunity. As early as 1927, Johnson (62) noted the frequent role of immunity in protecting flocks against coccidiosis outbreaks. He observed that resistance develops following "repeated inoculations with small numbers of oocysts." At the time of his death, he had begun experimental inoculations in hopes of developing a vaccination procedure. Although Farr (33) confirmed the significance of repeated exposure 16 years later, the clinical significance of this phenomenon was not fully realized until 1976, when Joyner and Norton (65) demonstrated the importance of very small numbers of oocysts in developing immunity. In a series of classic experiments, they demonstrated that daily inoculations of a single oocyst of *E. maxima* or small numbers of other species fed on 20 successive days produced stronger immunity than much larger numbers of oocysts given in a single inoculation. This phenomenon, which they named "trickle infection," is now recognized (22,60) as accounting for the naturally acquired immunity present in many flocks that have never shown signs of clinical coccidiosis. The reduced numbers of oocysts surviving in the litter continually reinforce any waning immunity initiated by early infections.

5. Longevity of oocysts. The omnipresence of viable oocysts makes a sterilization-eradication approach to control extremely difficult to achieve. Farr and Wehr (37) from the U.S. Department of Agriculture at Beltsville, Md., demonstrated that oocysts survive as long as 602 days in soil. Herrick *et al.* (50) reported the presence of live oocysts that survived for as long as 7 months in cecal tissue of live birds. Live oocysts are routinely recovered from almost all broiler houses in the United States (57,85). Immunity challenge studies also indicate the widespread distribution of oocysts because the majority of field flocks have proved to be immune, although they have experienced no signs of clinical coccidiosis (16,27,99). Thus, it may be generally concluded that oocysts are present wherever chickens have been introduced.

6. Resistance of oocysts to disinfectants. Oocyst walls are extremely resistant to many disinfectants commonly used against other types of pathogens. Johnson (62) discovered that potassium dichromate, which quickly kills bacteria and viruses, can be used routinely to preserve oocysts in the laboratory. Oocysts are resistant to formalin, quaternary ammonium compounds, copper sulfate, sulfuric acid, potassium hydroxide, potassium iodide, and potassium permanganate. Procedures used to sterilize poultry houses from other diseases are rarely successful with coccidiosis. The few oocysts that persist readily reinfect the next crop of birds as early as the first day after they are introduced.

CHEMOTHERAPY AND CHEMOPREVENTION

For the past 50 years, intensive study has centered on the discovery and development of chemotherapeutic and chemopreventive agents now extensively used by poultry producers. Because this subject has been reviewed by several authors in each of the eight succeeding editions of Diseases of Poultry (7,81,97), by Ryley and Betts (103), and by McDougald (82), only a few references will be cited here.

Early investigations. In 1934, Becker (6) enumerated use of some 36 compounds that had been tried unsuccessfully for therapeutic control of coccidiosis. Many of these were selected because of antimalarial, antibacterial, or other parasiticide activity. Use of buttermilk or other milk products (5) received extensive trials over a period of 20 years, but consistent control was not demonstrated under field conditions.

Sulfur. Considerable excitement was generated in 1935 at the American Society of Parasitologists meetings when Herrick and Holmes from University of Wisconsin (49) announced informally that feeding inorganic sulfur could prevent cecal coccidiosis. Feed levels of 10% to 20%, which could be tolerated only for short periods, were required to prevent mortality in

laboratory experiments. Proof of efficacy was established after comparing four groups of experimental birds: 1) medicated parasitized, 2) unmedicated parasitized, 3) medicated unparasitized and 4) unmedicated unparasitized controls (17). This design became the standard for evaluating efficacy of anticoccidials, thus eliminating false-positive results that can arise due to the self-limiting nature of coccidiosis. Currently, this experimental design is required in all efficacy studies.

Laboratory results with inorganic sulfur were confirmed in field trials (24,41,75), and some chemical companies began to market commercial preparations under such trade names as Cecagen®, Coxicurb®, and Coxitrol®. Although toxicity limited the practical use of sulfur, the discovery rejuvenated the search for a chemotherapeutic method of control.

The sulfonamides. In 1939, P. P. Levine (73) was credited with the discovery that sulfanilamide showed anticoccidial activity against the intestinal species *E. acervulina, E. praecox, E. mitis, E. hagani,* and *E. maxima.* Although inorganic sulfur showed some activity, sulfanilamide was ineffective against the important species *E. tenella* and *E. necatrix.*

The excitement in veterinary laboratories following the demonstration that protozoan diseases might be prevented or cured by chemotherapy was similar to that exhibited earlier in medical fields after the discovery that bacterial diseases could be arrested by using sulfonamides. As new sulfonamides were synthesized, intensive laboratory research was initiated comparing their activity against different species of bacteria and protozoa and on the physiology and pharmacodynamics of these drugs.

Levine (73), Farr and Allen (35), Horton-Smith (51), and Horton-Smith and Taylor (52) reported on the decreased output of coccidial oocysts following treatment with sulfonamides on infected chickens. Swales (108) believed that the coccidiostatic properties of sulfamezathine and sulfamerazine inhibited one or more stages of the life cycle as a consequence of weakening or destruction of the motile merozoites before penetrating other cells of the cecal epithelium. Farr and Wehr, using sulfamethazine (36), and Bankowski, using sulfaguanidine (2), showed that coccidiostatic action occurred upon the developmental schizogony stages of *E. tenella.* However, Waletzky and Hughes (115) concluded that the coccidiostatic action was dependent upon a relatively high drug level in the blood and ascribed little importance to the concentration in the intestinal tract.

At about the same time, new drugs, including arsenicals, and other classes of compounds were being synthesized and screened against bacterial and protozoan infections. Several drugs were found effective against *E. tenella,* and they became the forerunners of a new era in the pharmaceutical industry in which a prophylactic feed additive would become the primary method of controlling this poultry disease. To provide a team approach, the pharmaceutical industry employed chemists, parasitologists, veterinarians, nutritionists, advanced poultry producers, statisticians, and marketing specialists to discover and develop new anticoccidial drugs. Many of the best scientists from university staffs were employed or became consultants in this expanding industry.

Chemotherapy: treatment vs. prevention. A period of intensive research on various aspects of chemotherapy occurred between 1940 and 1952. Over 160 papers were published on testing anticoccidials in laboratory and field experiments (10,87). The need for discussing laboratory and field applications stimulated the first international conference on coccidiosis control (Table 1), sponsored by the New York Academy of Science (10). This conference, convened by parasitologist Sterling Bracket of American Cyanamid Co., brought together 51 scientists from three countries, 17 univeristies, six government institutions, and 10 pharmaceutical companies. Papers published during this period from university personnel included: Bankowski (3), University of California; Barber (4), University of Georgia; Bressler and Gordeuk (11), and Gordeuk and Thorp (43), Pennsylvania State University; Delaplane *et al.* (23), University of Rhode Island; Grumbles *et al.* (45), University of Rhode Island and Louisiana State University; Hawkins (48), Michigan State University; Herrick and Holmes (49), University of Wisconsin; Jungherr and Winn (66), University of Connecticut; Kay (68) and Koutz (69), Ohio State University; P. P. Levine (73), Cornell University; Peterson (93), Washington State University; and Seeger and Tomhave (104), University of Delaware. Papers from government institutions were contributed by Wehr and Farr (118), Farr (34), and Foster (39) from the U.S. Department of

Table 1. International conferences or symposia or coccidiosis.

Location	Date	Conference conveners	Chicken		Other birds[C]	Mammals[D]	Speakers U.S./ other countries	Pharmaceutical companies
			Immunity biology life cycle[A]	Use of drugs[B]				
New York	Nov. 1949	Brackett (10)	41%	41%	5%	14%	51/5	10
Chicago	Dec. 1959	Edgar (29) Hunter (54)	2	96	2	—	14/0	10
Athens, Ga.	May 1969	Reid (97)	0	100	0	0	31/4	10
Guelph, Canada	June 1973	Fernando *et al.* (38)	70	7	14	12	5/3	8
Tours, France	Sept. 1973	Yvore (119)	55	35	0	10	3/17	7
Nottingham, England	Sept. 1977	Long *et al.* (79)	60	37	2	0	10/11	12
Prague, Czechoslovakia	Nov. 1979	Bedrnik and Sevcik (8)	32	34	4	30	4/33	10
Pine Island, Ga.	Nov. 1985	McDougald *et al.* (86)	59	31	9	7	68/45	18
Tours, France	Oct. 1989	Yvore (120)	50	19	0.3	30	33/62	13

[A]Percentage of papers dealing with general biology, life cycles, and immunization in chickens.
[B]Percentage of papers dealing with use of anticoccidials in prevention or therapy in chickens.
[C]Percentage of papers concerning birds other than chickens.
[D]Percentage of papers concerning mammals.

Agriculture; Horton-Smith and Taylor (52) from the Animal Health Trust of Great Britain; and Swales (109) from the Department of Agriculture, Canada. Other contributions from the scientists employed by pharmaceutical companies are cited below in discussions of specific anticoccidials.

Answers to three key questions began to emerge:

1) Can the poultry industry afford routine use of these expensive anticoccidial drugs? Although some prognosticators in the early 1940s predicted use of drugs for chickens would never become financially feasible, large-volume manufacturing methods and competition for the anticoccidial drug market soon brought the cost down to less than one cent per bird.

2) Would chemotherapy for treatment or chemoprevention better serve the poultry industry? The prevention approach using medicated feed rather than treatment after diagnosis of the disease gradually became accepted by the poultry industry. Treatment after discovery of an outbreak and confirmation by laboratory diagnosis was almost always initiated too late to prevent mortality and morbidity losses. Anticoccidials show greatest protection if present in the feed early in the life cycle of the parasite. Some drugs must be present on the initial day of infection, whereas others show peak activity on days 2–4 of the life cycle. Signs such as intestinal bleeding, diarrhea, huddling, and anorexia do not usually appear until the fourth or fifth day of the life cycle. Not until many poultry producers had suffered severe losses using the treatment approach were they persuaded to use preventive medication as a form of insurance against losses from coccidiosis. A few producers in some parts of the world still elect to withhold medication until signs of the disease have appeared. In these cases, drugs showing activity late in the life cycle, such as sulfonamide or amprolium, are often used.

Most broilers are started and maintained on medicated feed until about a week before marketing. Drugs may then be withdrawn to save money during the final finishing period or because of regulations enacted by the U.S. Food and Drug Administration (FDA) to prevent harmful drug carryover into poultry meat. Young breeder and layer chickens are often similarly protected with anticoccidials, but the time of drug withdrawal varies with different management programs.

3) Which anticoccidial drug gives the best production at the least cost? Running debates have occurred at poultry, veterinary, and parasitology society meetings. To this day, such

discussions continue at frequent meetings. The colored charts diagrammed by McDougald and Reid (84) indicate estimates of the market share of drugs and show many yearly changes.

Pharmaceutical companies. With the continuing demand for anticoccidials by the poultry industry, officers of pharmaceutical companies were enticed by prospects for selling drugs by the ton rather than by the ounce. Substantial investments have been made to discover and develop new anticoccidials. Every year, thousands of newly synthesized organic compounds and fermentation products continue to be tested for anticoccidial activity using a blind screening program. If anticoccidial activity is detected, analogs may be synthesized in search of greater efficacy and/or less toxicity.

In vivo screening requires medicating three to 10 parasitized chicks and comparing their performance with unmedicated controls. *In vitro* screening was adopted after tissue-culture methods of growing coccidia on chicken embryo kidney or liver cells were developed by Patton (92) and Doran (26). This method of drug screening developed by Strout and Ouellette (107) and McDougald and Jeffers (83) has proved less expensive, and smaller quantities of chemical are required than with *in vivo* methods. After initiating these new methods, one company was able to increase screening tests from about 1000 tests per year in the 1960s to over 14 times this number in the 1980s. This company has completed over 200,000 tests since 1950. A second company has completed over 300,000 tests using both methods in the past 25 years. A third company, emphasizing the testing of synthesized analogs, has completed 60,000 screening tests, with about 2% of these products showing some anticoccidial activity. From this testing, not more than five or six marketable anticoccidials have emerged.

Although anticoccidial discoveries have come primarily from the study of chicken coccidiosis, drugs showing favorable responses have had further application for use on coccidia of turkeys, other birds, and mammals.

Pharmaceutical companies have frequently been called upon for support of educational activities connected with their products. Valuable educational and diagnostic aids in the form of films, books, and bulletins have been produced by Agri-Bio, American Cyanamid, Dow Chemical, Eli Lilly, Hess and Clarke, Merck, Norwich Animal Industry, Pfizer, and Salsbury Laboratories. Company representatives have frequently distributed a two-color diagnostic chart of nine species of coccidia from chickens originating at the University of Georgia (95). Edgar and Seibold (32) provided data indicating areas parasitized and characteristics of each species.

The broiler industry and new anticoccidial drugs. Broiler rearing as a specialized meat-producing industry originated in the Delmarva peninsula and Georgia in the late 1930s. By 1940, production had reached a plateau of 2 billion to 3 billion birds per year (USDA estimates, Fig. 1). At this time, coccidiosis was the most feared poultry disease, as flocks of 100 or more birds reared in confinement often suffered disastrous outbreaks. The accelerated increases in broiler production coincided with the availability of anticoccidial drugs that permitted larger flocks to be reared in a single house. Newer drugs have a wider range of activity against different species. In the 1940s, the ration had to contain 10–20% sulfur to control coccidiosis. The proportionate quantity of anticoccidial required in the feed has progressively decreased to less than 1 ppm for a new drug now in late stages of field testing. Sales of anticoccidials used largely for broilers in United States increased from an estimated $800,000 in 1957 to $15 million in 1967, $50 million in 1977, and $83 million in 1987. Similar developments in broiler production in Europe and in other parts of the world have resulted in a total market demand of $300 million.

Marketing successes with anticoccidials. Less than half of 24 anticoccidials introduced in the United States during the past 40 years (Fig. 1) would be considered commercial successes. Others would be listed as complete failures. Between 1948 and 1955, three or four anticoccidials became widely used in broilers. Sulfaquinoxaline (SQ®), which has activity against *E. tenella* in the laboratory (23) and in field flocks (45), was widely used in broilers between 1948 and the mid 1950s. SQ has recently been withdrawn from the market due to a long withdrawal requirement before slaughter, widespread emergence of drug-resistance, and some concern with mild nephrotoxicity.

Nitrophenide (Megasul®) was introduced by Waletzky *et al.* of American Cyanamid Co. (116) in 1949 and used largely as a broiler anticoccidial. Nitrofurazone (NFZ®), one of the nitro-

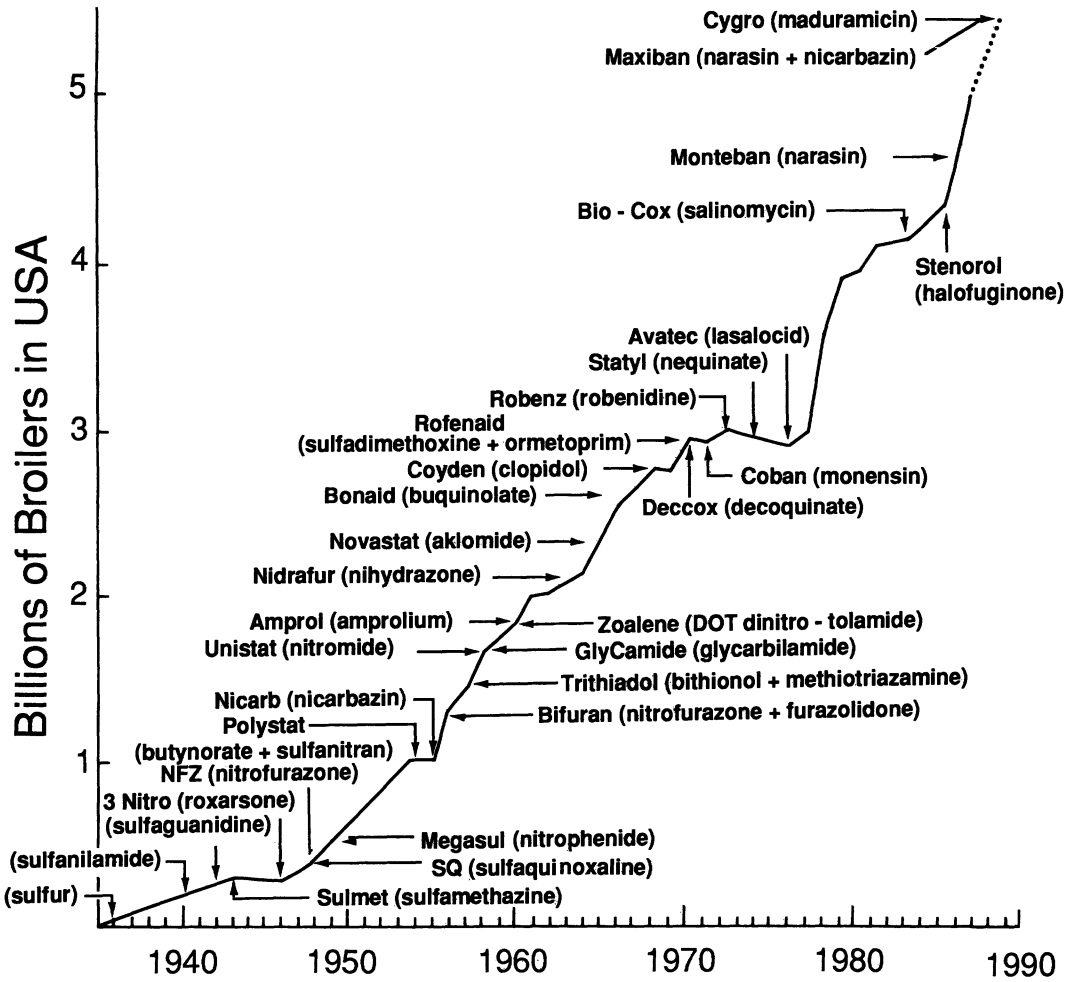

Fig. 1. Anticoccidial drugs introduced in the United States during 1936–89 plotted on USDA estimates of numbers of broilers produced. Registered trade names begin with a capital letter and generic names are shown in lower case.

furans, was also successfully introduced in 1949. Although Norwich Pharmacal Co. controlled the patents for the nitrofurans, the attempt to market this and other drugs on a veterinary prescription basis was not widely supported by the poultry industry. Harwood of Hess and Clarke (47) was able to directly reach the poultry market through feed industries without prescription requirements.

Cuckler *et al.* of Merck, Sharp, and Dohme Laboratories introduced nicarbazin in 1955 (19). This drug is a molecular complex of two chemicals, with the combination greatly enhancing anticoccidial activity. Because this drug has had few problems with emergence of resistant strains, it is still being used for broilers. In 1960, amprolium was released (Ott *et al.*) (91). Although widely used for broilers, absence of drug carryover into commercial eggs has permitted its use on layers throughout the life of the bird. Such long-term use is seldom practiced because of the expense and the lessened risk of coccidiosis outbreaks in older flocks that may have developed partial immunity from natural subclinical infections.

Two dinitrobenzamides with similar chemical structures and activity against *E. tenella* and *E. necatrix* were discovered almost simultaneously by Morehouse and McGuire (90) of Salsbury Laboratories and by Hymas and Ste-

venson (55) of Dow Chemical Co. Salsbury's Unistat® was marketed in combination with two other active ingredients. Dow's Zoalene® differed from Salsbury's dinitrobenzamide by a single added methyl group. Salsbury received marketing approval in 1958 from the FDA and soon captured much of the broiler market. Marketing approval of Zoalene was delayed for two more years while the FDA awaited additional data on possible residues.

Monensin, the first anticoccidial manufactured by fermentation technology, was commercially introduced in 1971 by a team at Eli Lilly & Co. headed by Ray Shumard and Maury Callender (106). Monensin, representing a new class of anticoccidial known as an ionophore, proved to have less tendency to develop drug resistance than many older anticoccidials. Its immediate commercial success far surpassed all company and poultry industry expectations, to the extent that it was placed on allocation for three years while fermentation manufacturing facilities were enlarged fourfold. It dominated the world market for 12 years and still shares a significant part of the market with other ionophores.

Lasalocid, another ionophore produced as a fermentation product, had been discovered in 1951 but was not introduced as a poultry anticoccidial until 1974 by Mitrovic and Schildknecht (88). In Japan, a third ionophore, salinomycin, with good anticoccidial activity was developed by Tanaka *et al.* of Kaken Chemical Co. in 1973 (110). It has been approved and successfully marketed by four major pharmaceutical companies in different parts of the world. Approval by the FDA for use in the United States was delayed until 1983. Another ionophore, maduramicin (Cygro®), which is efficacious at 5–6 ppm, was discovered in the United States by Kantor and Schenkel in 1984 and developed by American Cyanamid Co. (67). It was first introduced in numerous countries overseas before attempting the more extensive testing required for approval in the United States. Marketing was granted by the FDA in 1989, but marketing plans have been delayed awaiting combination clearances for use with roxarsone and other desired feed additives.

Several promising new organic chemicals and ionophores are currently being field-tested with the hope that they will eventually be approved for commercial use as anticoccidials.

Combinations and synergism. Combinations of two or more drugs have often been marketed to enhance the species spectrum of activity or to provide other beneficial properties. Two early examples are Polystat®, containing four anticoccidial ingredients, and Unistat®, containing three anticoccidial ingredients, marketed by Salsbury Laboratories. Both contain the sulfonamide sulfanitran and the organic arsenical roxarsone (3 Nitro®) to enhance growth and for increased activity against *E. acervulina* and other species. Although roxarsone by itself has some anticoccidial activity against *E. tenella,* it is approved only for "growth promoting" properties. Polystat contains the organic tin compound butynorate, which was originally discovered as an anthelmintic. Both of these combinations have been discontinued because their market penetration could not justify developing the data to comply with newer FDA guidelines. Butynorate (Tin-O-Stat®), the primary anticoccidial ingredient of Polystat, is still being marketed by itself for turkeys. Another example of a combination was the addition of ethopabate to enhance the activity of amprolium against *E. acervulina.*

Several combinations have been developed using a pyrimidine compound plus a sulfonamide. The combination provides biochemical activity due to inhibition of folic acid production at two points in its synthesis. One such combination marketed by Hoffmann-LaRoche (89) under the trade name Rofenaid® is priced at twice the cost of other anticoccidials. This high price has limited its use to prestarter feeds where the product may also provide antibacterial as well as anticoccidial activity.

Narasin plus nicarbazin shows a true synergistic combination as described by Callender and Jeffers (14). This combination has recently been approved for marketing. At low levels, both drugs combined show greater efficacy than either drug alone at their higher recommended levels. The combination has the advantage of overcoming borderline toxicity that is sometimes shown at higher levels, and it is particularly effective against those isolates that have developed drug resistance to either or both of the components.

Government regulations, guidelines, and FDA clearances. As early as 1949, the FDA was involved in setting standards and requiring approval for use of drugs in poultry feed, as well as for other livestock (17). At this time, approval

was sometimes granted within a few hours after presentation of requested data from the pharmaceutical company. With improved chemical sensitivity tests for detecting possible residues in poultry meat, requirements for testing were multiplied by the growing staff of the FDA, which now includes chemists, pathologists, veterinarians, parasitologists, toxicologists, statisticians, and legal experts. Added requirements have often resulted in expensive delays due to miscalculated marketing plans. The assigned task of the FDA officials in examining the great stacks of experimental data cause further delays. Required paper reports presented to the FDA have sometimes been calculated by weight, with data submitted on one drug weighing over 80 pounds. Pharmaceutical representatives now regard the task of securing approval for a new drug more difficult than its initial discovery, manufacture, and development.

Regulatory requirements continue to necessitate additional testing and expense in the development of an effective anticoccidial. Extensive laboratory investigations conducted in batteries must demonstrate efficacy individually and in combinations against six pathogenic species of recent field isolates obtained from various geographic areas. Less-frequent isolations of *E. necatrix* and *E. brunetti* from broiler flocks during recent years have made it necessary to find these species in breeder or layer flocks. Other requirements include: target animal toxicology studies in floor pens at the recommended use level and mutliples of this level; testing with induced *Salmonella* infections to determine the effect on the incidence of tissue retention, fecal shedding and resistance patterns to a number of antibiotics and organic chemicals; testing with *Escherichia coli* infections to determine the effect on resistance transfer factors; floor-pen studies in selected geographic aras to illustrate efficacy and safety; compatibility studies with other feed additives demonstrating lack of effect on the efficacy of each feed additive; and absence of detectable residues of the anticoccidial or its metabolites after both battery and floor-pen experiments.

In 1970, both the poultry industry and the pharmaceutical industry welcomed the appointment of Thomas Raines to the FDA as veterinary medical specialist and consultant. His previous experience in academic and diagnostic laboratories, and as a public health veterinarian, livestock inspector, and with the development and marketing of the anticoccidial buquinolate with the Norwich Pharmacal Co. provided a strong background for his assignment with FDA. He has been entrusted with both writing and interpreting the guidelines for poultry, game-bird, and rabbit anticoccidials. This assignment included planning with pharmaceutical companies for needed experiments. Although Raines has been diligent and uncompromising in his protection of the consumer, the environment, and the health of the medicated species, he has simultaneously assumed that it was his duty to see that products that would benefit the poultry industry would receive fair and rapid review under current regulations. Raines retired in 1989. His assistance will be missed by all associated with coccidiosis control programs.

Unexpected delays with the FDA due to increasing requirements have raised estimates of the cost of discovery and approval for a single new product to $21 million. Salsbury Laboratories, Norwich Pharmacal Co., Dow Chemical Co., and Imperial Chemical Industries, Ltd. (Great Britain), have withdrawn further risk capital in the search for new anticoccidials. Others have elected to test preliminary marketing success of promising anticoccidials in other countries before developing the data required for approval in the United States.

In retrospect, poultry producers should be thankful for the protection afforded by FDA regulations in this day of increased consumer activism. The increasingly restrictive requirements have provided double assurance that the human food supply is not contaminated by chemicals used in control of coccidiosis in poultry. Many other countries have set guidelines similar to those required in United States before granting marketing approval.

Feed manufacturers. The demand for medicated feed often places feed manufacturers in charge of selecting an anticoccidial drug and thus making the feed companies responsible for failures in coccidiosis control programs (18). Shortly after the introduction of nicarbazin, many feed manufacturers faced special problems associated with accidental contamination of layer feeds with this broiler anticoccidial. The pharmaceutical company (Merck) had provided adequate warnings that hens would lay mottled eggs or go out of production if accidentally fed

low levels of the drug. Lawsuits were usually settled out of court in favor of the grower, but there was not always proof that nicarbazin contamination had caused the problem. Because of the electrostatic properties of nicarbazin, layer feeds sometimes became contaminated from equipment previously used for mixing broiler feeds. Separate feed mills were sometimes built for the exclusive purpose of preparing uncontaminated breeder and layer feeds.

Conferences and symposia on coccidiosis control. Coccidiosis control has been the subject of frequent seminars and conferences. Some of these were sponsored by pharmaceutical companies after new anticoccidials had received government approval for marketing. In some cases, guest lists were limited to possible users. Data presented were sometimes selected to promote sales. In other cases, an open scientific approach was followed, and both favorable and unfavorable data on the new anticoccidial were presented. Paul Harwood (47), a parasitologist with Hess and Clarke, arranged a series of three open seminars on the nitrofurans held successively at Michigan State University and the universities of Georgia and Kentucky. This series is now remembered more for the negative criticism presented by some invited scientists than for the benefits of and precautions for using furazolidone in control of coccidiosis and salmonellosis.

More lasting information on coccidiosis came from scientific meetings sponsored as open international conferences held under auspices of noncommercial agencies (Table 1). They were generously supported financially by seven to 18 pharmaceutical companies. The printed papers and discussions presented at these conferences greatly advanced the technology for developing all methods of coccidiosis control.

As intensive broiler production methods were adopted in other parts of the world, an increasing number of scientists from abroad participated in these conferences. Different types of organizations outside the United States have hosted these seminars in Canada, England, Czechoslovakia, and France.

One early conference originated and dealt entirely with unexpected problems of feed manufacturers at the time nicarbazin was first introduced. The American Feed Manufacturers Association scheduled a two-session conference (54) to suggest standards for the release of new anticoccidials for the poultry industry. Edgar (28) summarized a list of 17 characteristics of a good anticoccidial: Besides showing efficacy against all economically harmful species, anticoccidials and premixes should not be toxic, hygroscopic, or electrostatic. They should not cause excitability, impair production, hatchability, or feed conversion, or be harmful in any way to animals or humans. Although the perfect anticoccidial drug may never be found, conference participants set goals for pharmaceutical manufacturers. Discussions occurred during these meetings for improving methods of evaluating anticoccidial drugs and the species against which efficacy was required.

The 1969 conference at the University of Georgia (96) was arranged at the request of several pharmaceutical companies to discuss new FDA guidelines for approval of anticoccidials. The usefulness of floor-pen experimentation with simulated coccidiosis outbreaks as presented by Brewer and Kowalski (12) and Cover (18) became fully recognized after this meeting.

More recently, a higher percentage of papers in these conferences have dealt with the biology of the coccidia (8,38,79,86,119,120). An increasing interest in vaccination and immunity is reflected in titles of papers in more recent conferences.

Drug resistance. Besides the continual expense of using drugs, the major drawback to this method of coccidiosis control has been the emergence of resistant strains after the drug has been used in the field. Although this phenomenon has long been recognized and was recently reviewed by Chapman (15), drug manufacturers have been unable to predict the useful market life for newly introduced anticoccidials. Manufacturers of Glycamide® and several promising quinolines, e.g., Bonaid® and Statyl®, completely lost their developmental costs when resistance appeared within weeks or months of marketing. Another quinoline compound, decoquinate (Deccox®), was successfully marketed by Hess and Clarke after sampling and pretesting for resistance samples of oocysts that had been isolated from the premises of prospective customers. Drug sales were limited for use on premises where the absence of strains resistant to the quinolines had been demonstrated (57).

The marketing success of monensin was due in part to a fortunate release date in the early 1970s. Knowledgeable poultry producers had become pessimistic about using chemical control methods for coccidiosis. Resistance had been noted for all of the currently available drugs including Amprol®, Zoalene®, Coyden®, Unistat®, and the quinolines. Various methods of prolonging their usefulness had been instituted, including use of shuttle and drug-rotation systems. Monensin controlled these resistant strains while strains resistant to this anticoccidial were slow to emerge during its use. Enthusiastic users stated that they could now forget about coccidiosis as a poultry problem. After some 18 years of monensin use, partially resistant strains of both chicken and turkey coccidia have become more common. These strains may also show partial cross-resistance to the other polyether ionophores, lasalocid, salinomycin, and maduramicin.

SANITATION AND DISINFECTION AS A CONTROL METHOD

Before methods involving chemoprevention of coccidiosis were available, textbook literature contained many suggestions for prevention using various sanitary measures. With one exception, these have proved impractical or unsuccessful with the modern poultry industry. This exception relates to the use of wire floors or cages, which prevent birds from contact with feces contaminated by oocysts. Advocates of cage operations often cite coccidiosis control as a reason for adopting this method of poultry management. Long ago, poultry producers learned the hard way that birds that were started in cages and were still susceptible could not be safely moved to floor management without risking coccidiosis outbreaks. They were usually unaware that floor-reared birds of the same age had acquired some subclinical exposure and developed partial immunity. Numerous outbreaks in caged birds have been reported (13,40). Such outbreaks often occur in a single line of cages where feces had contaminated a common source of feed or water. For cage management, continual vigilance is required to see that fecal disposal systems operate properly.

Since Tyzzer and Johnson first noted the resistant nature of coccidial oocysts, a search for suitable disinfectants to kill oocysts has been continued by a legion of research workers. Over 130 papers are cited by workers originating in 11 or more countries, including Egypt, France, Great Britain, Hungary, Italy, Japan, the Netherlands, Poland, the Soviet Union, the United States, and West Germany.

In experimental coccidiosis laboratories where inactivation of all oocysts is essential, a few relatively toxic and sometimes hazardous disinfectants have been used. Cresylic acid compounds, ammonia as a gas or liquid (53), and methyl bromide as a gas (9) have found limited use. Every laboratory worker can testify that sterility from oocysts is difficult to maintain. Many experiments have been nullified by accidental infection due to incomplete sterilization (98). Outbreaks have occurred in attempts to produce specific-pathogen-free or germ-free chickens.

Attempts to rear oocyst-free flocks in floor pens under commercial conditions have similarly been unsuccessful. Coccidiosis outbreaks have often occurred in new houses in which birds have never previously been reared. Such outbreaks have been informally named "the new-house coccidiosis syndrome." This syndrome has occurred due to early absence of oocysts that usually initiate accidental exposure resulting in some immunity. An introduction of oocysts late in the life of a flock may occur at a time when moist litter and warm temperatures permit a rapid buildup of oocyst numbers. This sudden exposure results in a severe outbreak in the completely susceptible flock. Such occurrences suggest that attempts to provide complete sterility from oocysts may be counterproductive because they prevent subclinical coccidiosis to fortify the flock with immunity. Poultry can be reared oocyst-free in floor pens with extreme vigilance by providing filtered air, preventing contamination of feed and water, and requiring workers to change clothing on entering pens. Such procedures are generally regarded as impractical for commercial enterprises.

Another approach to using disinfection for coccidiosis protection would be an attempt to markedly reduce the number viable oocysts in the pens, thus precluding attacks of clinical coccidiosis. Unfortunately, clean-out and "terminal disinfection" (44) occurs when very few oocysts are present in the litter. In numerous studies of broiler houses, it has been shown that numbers

of oocysts peak at 4–5 weeks of age and very few oocysts survive by the time the birds are ready for market (100). Although use of certain disinfectants in poultry houses is sometimes recommended as a method of coccidiosis control, such a recommendation is highly debatable.

Conclusions on disinfection as a control measure. A restudy of Tyzzer's (114) and Johnson's (64) suggestions may be useful. Tyzzer stated in 1932 that, "Attempts to rear chickens in the absence of all coccidial infection are in general ill-advised, and the gradual building up of immunity through repeated light infections appears to furnish more promise." Similarly, Johnson concluded, as indicated in his posthumous publication (64) that poultry producers using conventional rearing methods "should not be encouraged to attempt raising fowls to maturity free of coccidial infections."

RESISTANCE AND IMMUNITY

Innate resistance. There have been numerous studies indicating that some strains of birds show partial resistance to coccidiosis, as reviewed by Jeffers and Shirley (59). This innate resistance, which is genetically controlled, needs to be distinguished from naturally acquired immunity induced by oocyst exposure. Although coccidiosis losses could be at least partially ameliorated by selective breeding for coccidiosis resistance (31), breeding organizations have not found such a program profitable (42,46).

Naturally acquired immunity. Since the pioneering work of Johnson and Tyzzer, researchers have recognized the important role of naturally acquired immunity in protecting older flocks against coccidiosis losses. As new anticoccidials were released, various studies have been made on effects of their use on development of flock immunity. Because all drugs may cause some suppression in oocyst development, their use may affect the numbers of oocysts present and thus severity of a challenge dose on poultry flocks. However, no drug has been discovered that will completely suppress all oocyst production under commercial conditions. Thus, use of drugs occasionally influences the speed at which flock immunity to certain species develops. Some programs have recommended gradual decreases in drug levels to assist in development of natural flock immunity. Because older non-immune birds remain fully susceptible to coccidiosis, the timing of drug withdrawal may become a critical decision of the poultry producer.

Planned immunization. There have been many attempts to devise artificial methods to vaccinate baby chicks in field flocks. Although birds are readily immunized by feeding a measured dosage of oocysts under laboratory conditions, controlling dosage with the many species involved under field conditions presents a more difficult management problem.

Between 1948 and 1959, about 95% success was achieved in field flocks on the west coast by Dickinson *et al.* (25), who administered measured doses of five species of oocysts in the feed, followed by careful timing with sulfaquinoxaline treatment 24–36 hours later. Each species required carefully controlled but different dosages in order to produce flock immunity. Although good flock immunity resulted, limiting factors preventing widespread adoption were: the cost of producing enough oocysts of all species to provide protective immunity with a single inoculation and the necessity for very close supervision on timing for both inoculation and treatment.

A less-expensive program in terms of oocyst requirements was initiated by Edgar (29) and Edgar and King (31). Small, but programmed numbers of oocysts of several species are fed via feed or drinking water. These mild infections seed the litter with a second generation of oocysts. Daughter generations of oocysts continue to reinforce immunity by means of trickle infections. This planned immunization program has been extensively used with valuable breeder stock. Oocysts of eight species commercially prepared under the name of CocciVac® are administered in water or fed to birds at about 10 days of age. Litter moisture must be controlled to permit optimum sporulation of daughter oocysts. Although the large numbers of oocysts occasionally produced by second- or third-generation life cycles may produce mild pathogenesis, treatment with anticoccidial drugs is seldom recommended. Failures to produce good immunity with this program are largely attributed to insufficient care of the vaccine or poor litter management.

Edgar (30) has described a similar planned immunization program for turkeys in United

States, and Lee (70) has developed a program for vaccinating turkeys and roaster chickens in Canada. Although planned immunization programs for broiler production have often been successfully demonstrated, they have not been widely adopted commercially. Protection is provided more conveniently with less management supervision by administering anticoccidials in the feed.

PROMISING RESEARCH ON VACCINATION AND IMMUNITY

For coccidiosis, as with other vaccination programs, the producer hopes for a flock delivery system that does not require handling of individual birds. Davis and Harris (21) have described a convenient system of administering measured numbers of oocysts in calcium alginate pellets in feed. Numerous papers describing this method have been reviewed at the last two international symposia (86,120). The economics of manufacturing and large-scale field use have yet to be fully demonstrated.

Another experimental method demonstrated by Bafundo (1) is to administer oocysts of one or more species to 1-day-old chicks in the hatchery using a Beak-o-Vac® machine or by spraying a suspension of oocysts over chicks in a closed container. In the latter case, a larger number of oocysts is required to assure that some are swallowed during preening.

For many years, attempts have been made to find or produce attenuated strains of coccidia for use in vaccination procedures. Jankiewicz and Scofield (56) used heat treatment of oocysts. Waxler (117) used x-ray irradiation to produce attenuated strains. These methods did not produce genetically reproducible strains. True genetic attenuation has been achieved by two methods: 1) serial passage of strains in parasitized chick embryos, as described by Long (77); and 2) selecting for precocious strains, as originally described by Jeffers (58). The latter method shows greatest promise for developing a true vaccine strain.

The mechanism by which a distinctive cellular immunity produces protection after humoral antibodies have disappeared from the blood stream has been a topic of investigation for many years. In spite of recent advances in the field of immunology, many of the basic mechanisms remain a mystery. Reviews of the present knowledge of immunology to coccidiosis have been published by Rose (101,102) and Long (78).

As reviewed by Danforth (20), recent progress has been made in understanding the basic nature of coccidiosis and immunity using techniques of genetic engineering. These studies will enhance the understanding of this disease complex. Although several pharmaceutical companies and government agencies have research goals of producing a useful vaccination program, practical application appears to be some years away. Popular press releases have probably generated premature excitement about practical vaccination procedures involving new techniques for use in the poultry industry.

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